## P1160 - CHITOSAN/DNA NANOPARTICLES CHARACTERISTICS DETERMINE THE TRANSFECTION EFFICACY OF GENE DELIVERY TO HUMAN MESENCHYMAL STEM CELLS

***Elham Malakooty*[1,2]*, Mohamadreza Baghaban Eslaminejad*[1]*, Nematollah Gheibi*[2]*, and Fatemeh Bagheri*[1,3]

[1]Department of Stem Cells and Developmental Biology at Cell Science Research Centerstem cell, Royan institute for Stem Cell Biology and Technology, ACECR, Tehran, tehran, Islamic Republic of Iran; [2]Cellular and Molecular Research Center, Qazvin university of medical sciences, Qazvin, Qazvin, Islamic Republic of Iran; [3]Department of Cell and Molecular Biology, University of Tehran, Tehran, Tehran, Islamic Republic of Iran

**Purpose:** The objective of this study was to evaluate the potential of prepared chitosan -plasmid DNA nanoparticles in transferring the exogenous gene into human bone marrow-derived mesenchymal stem cells. **Methods:** Chitosan/pDNA nanoparticles were synthesized through the complex coacervation method using 18, 50 and 136 KD chitosan and pTracer-CMV2 plasmid containing Green Fluorescent Protein (GFP) gene. In this regard several concentrations of chitosan solutions (0.05%, 0.1%, 0.5% and 1%) were used. To examine the complexation, samples were run through an agarose gel. The size and zeta potential of nanoparticles were measured by Nanosizer. Scanning electron microscopy (SEM) imaging was used to observe the morphology of nanoparticles. MSCs were prepared from human bone marrow and transfected with chitosan/pDNA nanoparticles. The cultures transfected by lipofectamine2000 was taken as the control. Cell viability and transfection efficiency were determined by MTT assay and flow cytometric analysis respectively. **RESULTS:** The smallest size of complexes was obtained using 50KD chitosan (about 50nm) and the highest zeta potential was with 136 KD chitosan (29.61mV). The best transfection rate (18.43%) was achieved with 0.1% concentration of 18KD chitosan nanoparticles. commercial lipofectamine transfected 40.57% of cells. MTT assay indicated an average of 95.5% cell viability for 0.1% concentration of 18KD (chitosan compared with about 60% of Lipofectamine2000. **Conclusions:** Nanoparticles produced by 18KD chitosan at 0.1% concentration and pDNA would be a promising gene delivery system to human marrow derived MSCs. Although transfection efficiency of such nanoparticles is lower than that of Lipofectamine2000 but they comparatively possess less cytotoxic effects.

**Keywords:** Chitosan, pDNA, Nanoparticle, Mesenchymal stem cell, Transfection

## P1161 - PLATELET HARVEST, CRYOPRESERVATION AND INFUSION; RE-EXPANDING THE RESOURCES OF THE BMT LAB

***Christine Hicks****, Nicole Goodman, Rosalie Gemmell, Fernando Roncolato, and Sundra Ramanathan*

St George Hospital, Sydney, New South Wales, Australia

A 53 year old multiparous woman with a hepatic mass was found to have AML. She proceeded to induction chemotherapy resulting in complete remission by day 28. Between cytarabine consolidation cycles, she received 3 TACE procedures for presumed hepatocellular carcinoma. During first cycle, she developed platelet refractoriness with no platelet increment one hour post transfusion. Testing confirmed the presence of an HLA antibody. HLA matched platelet transfusions did not give a satisfactory platelet increment. The decision was made to harvest and cryopreserve her platelets, then reinfuse post chemotherapy.

The patient was scheduled to have plateletpheresis with a goal of $10 \times 10^{11}$ platelets to be harvested and cryopreserved, allowing for 4 infusions as support over the nadir post-chemotherapy. This was to be repeated to support the final round of chemotherapy.

**Collections:** Once platelet count reached > 150, 4 collections were performed over 2.5 months yielding between 3.1 to 5.5 $\times 10^{11}$ platelets per harvest, irradiated at 25Gy, and cryopreserved in 5% DMSO using a controlled rate freezer. Studies on her post-thaw platelets using PRP impedance indicated a 50% reduction in functionality compared with fresh platelets. **Infusions:** Four infusions were performed 11-20 days post consolidation 2 chemotherapy and three infusions 11-19 days post consolidation 3. No adverse reactions were reported. Platelet counts increased typically from 11-29 (1.6 to 2.6 fold) within 24 hours of infusion. HLA matched platelets gave no increase (pre-infusion count of 8, to post of 9, within 24 hours). There were no clinically significant bleeding complications and she was able to finish her scheduled rounds of chemotherapy. **Conclusions:** We have presented a case study requiring harvest, cryopreservation and infusion of autologous platelets. Incorporating archived platelet protocols into current practices was easily and safely achieved by the BMT laboratory. The methodology, equipment and processes involved required little change from the traditional role of progenitor cell work and this can safely extend the services offered by BMT laboratories with minimal additional costs for equipment or consumables.

## P1162 - BONE FORMATION AND THE DEVELOPMENT OF BONE MARROW

***Miriel Ho*[1,2]*, Hatem Salem*[1]*, Stephen Livesey*[2]*, and Kathy Traianedes*[2,3]

[1]Clinical Haematology, Monash University, Melbourne, Victoria, Australia; [2]Clinical Neuroscience, St Vincent's Hospital, Melbourne, Victoria, Australia; [3]Medicine, The University of Melbourne, Melbourne, Victoria, Australia

**Background:** Lifelong haematopoiesis is mediated by haematopoietic stem cells (HSCs) that give rise to all blood lineages. Long-term quiescent HSCs localise to the interface between bone and bone marrow (B-BM). This anatomical juxtaposition belies a functional relationship between bone metabolism and haematopoiesis. The mature B-BM interface is a complex structure composed of intricate networks connecting cells, extracellular matrix, and growth factors. This complexity renders investigation of the component parts of the B-BM interface extremely difficult. **Aim:** This study uses a unique model to investigate the early spatial and temporal events leading to B-BM formation. **Methods:** Rat acellular demineralised bone matrix (DBM) was implanted intermuscularly into the hind-limbs of nude mice, and explanted between days 2-28. The resultant ectopic nodules were subjected to macroscopic, histological and morphometric analyses (microCT scanning), gene expression studies and colony-forming unit (CFU) assays. **Results:** The progressive increase in vascularity and hardness of the nodules was reflected in their corresponding histological analyses. Neovascularisation was an early event and appears to be essential for cellular repopulation of the DBM. MicroCT imaging of explanted nodules first detected X-ray dense areas by day 10. This preceded the appearance of haematopoietic progenitors evident first on day 14 (CFU assays). The same types of CFUs were cultured from the corresponding long-bone marrow. DBM remodelling resulted in mature nodules consisting of newly formed bone (with the presence of osteoblasts, lining cells, osteocytes and osteoclasts) encompassing a centralised marrow cavity; which was confirmed histologically. **Conclusion:** Collectively, these findings suggest that bone is integral to, and precedes the development of marrow. On-going studies will determine critical changes in gene expression between days 7-14 that may be associated with bone marrow development. The use of this unique ectopic bone formation model to study the genesis of haematopoiesis may give us specific insights into critical parameters that can influence microenvironmental cues for HSCs.

## P1163 - P18INK4C IS AN UNIQUE TARGET FOR HEMATOPOIETIC STEM CELL EXPANSION WITH SMALL MOLECULE INHIBITORS

***Yingdai Gao*[1]*, Hongmei Shen*[3]*, Peng Yang*[2]*, Hui Yu*[3]*, Zhaojun Xie*[2]*, Liyan Zhang*[1]*, Patrick Bartlow*[2]*, Qing Ji*[1]*, Yahui Ding*[1]*, Lirong Wang*[2]*, Sha Hao*[1]*, Shihui Ma*[1]*, Fang Dong*[1]*, Yanxin Li*[1]*, Peng Zhang*[2]*, Haizi Cheng*[2]*, Weimin Miao*[1]*, Youzhong Yuan*[3]*, Tao Cheng*[1]*, and Xiang-Qun Xie*[2]

[1]State Key Laboratory of Experimental Hematology, Institute of Hematology and Blood Diseases Hospital, Tianjin, China; [2]Pharmaceutical Sciences, Drug Discovery Institute Pittsburgh Chemical Methodologies & Library Development Center, Pittsburgh, Pennsylvania, USA; [3]Radiation Oncology, University of Pittsburgh School of Medicine and University of Pittsburgh Cancer InstituteBlood Diseases Hospital, Pittsburgh, Pennsylvania, USA

The G1-phase of cell cycle is a critical window in which stem cell self-renewal may be balanced with other fate choices. The cyclin-dependent kinase inhibitors (CKIs), including p15, p16, p18, p19, p21, p27 and p57, have been reported to be involved in the regulation of stem cells especially the hematopoietic stem cells (HSCs). Among all the CKIs examined in HSCs, p18 and p27 were shown to have inhibitory effects on HSC self-renewal or repopulation in mice. In this study, we show that p18 is a more potent inhibitor for HSC self-renewal than p27 in vivo. Absence of p18 allowed HSCs to be expanded and maintained in a long-term culture system for more than 15 weeks. Surprisingly, overall cycling of hematopoietic cells was not increased in the absence of p18. Instead, a favored outcome of self-renewal after HSC division was indicated by single cell analyses. To target p18 pharmacologically, we have designed a number of p18 small molecule inhibitors via in silico-screening and validated them by functional assays. Importantly, the identified lead compounds were able to specifically expand HSCs in vitro. Thus, these novel p18 small molecule inhibitors offer valuable chemical probes for dissecting the signaling pathways of self-renewal of tissue stem cells, and serve as important lead compounds to further develop more effective agents for therapeutic stem cell expansion.